bio-protocol Preprint

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Preparation of cell extracts

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An abbreviated version of this protocol was published in Life Science Alliance in Feb 2022

Visualized procollagen Iα1 demonstrates the intracellular processing of propeptides

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Detailed protocol

Forty-eight hours after transfection, cells in 35 mm dish were washed twice with PBS and lysed with 50 µL of EBC lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, and 0.5% NP-40) supplemented with an inhibitor mix (1 ng/ml of aprotinin, 100 mM β-glycerophosphate, 1 mM NaF, 1 mM Na3VO4, 10 µg/ml of leupeptin, 10 µg/ml of pepstatin A, and 1 mM phenylmethylsulfonyl fluoride) or 50 µL of NativePAGE sample buffer (Thermo Fisher Scientific) supplemented with 10% n-dodecyl-β-D-maltoside and the inhibitor mix described above. The cell extracts were cleared by centrifugation at 15,000 rpm for 15 min at 4 ºC. The protein concentration of the precleared extracts was measured with Pierce BCA protein assay kit (Thermo Fisher Scientific).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Tanaka, T. and Moriya, K. (2022). Preparation of cell extracts. Bio-protocol Preprint. bio-protocol.org/prep1797.
2. Tanaka, T., Moriya, K., Tsunenaga, M., Yanagawa, T., Morita, H., Minowa, T., Tagawa, Y., Hanagata, N., Inagaki, Y. and Ikoma, T.(2022). Visualized procollagen Iα1 demonstrates the intracellular processing of propeptides. Life Science Alliance 5(5). DOI: 10.26508/lsa.202101060